Tandem Supercritical Fluid Extraction/Chromatographic Studies of the Desert Botanical Species, *Dalea spinosa*

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Abstract. Off-line and on-line supercritical fluid extraction (SFE) coupled with gas chromatography (GC), supercritical fluid chromatography (SFC), and mass spectrometry (MS) have been used to characterize the composition of extracts from the desert botanical species, *Dalea spinosa*. Off-line SFE conducted at pressures as high as 680 atm yielded odoriferous extracts similar in composition to an isopropyl alcohol-derived extract when the two extracts were characterized by SFC and GC/MS. SFC of the extracts revealed the presence of up to 18 components, GC/MS indicated six major compounds, and headspace GC/MS showed over 20 components. On-line micro-SFE/SFC was performed on single seeds of *D. spinosa* as well as dissected single seeds over the pressure range of 100-400 atm at 45°C. SFE/SFC on portions of a single seed, such as the calyx or incised resin sac, indicated subtle differences in molecular composition. These results suggest that the resin sac is particularly enriched in many odoriferous compounds.

Key words: *supercritical fluid extraction, supercritical fluid chromatography, Dalea spinosa, desert smoke tree*

INTRODUCTION

Supercritical fluid extraction (SFE) using carbon dioxide is a particularly appropriate processing technique for use in the food and cosmetic industries. The physiologically inert nature of CO_2 yields uncontaminated extracts which have considerable appeal to health conscience consumers. In addition, extraction with supercritical CO_2 can yield extracts having enhanced flavor/odor characteristics and compositions similar to those occurring naturally in the botanical substrate.

SFE has been used to advantage in studying the volatile and semi-volatile components in a number of botanical materials [1-7]. The technique offers a viable and benign alternative to the traditional isolation methods, such as steam distillation and liquid solvent extraction, and the analytical methodologies of headspace and purge-and-trap. These methods frequently use large volumes of organic solvents, require several hours to perform, may yield low extraction efficiencies, and can result in the loss or degradation of the analytes [8-9].

The desert smoke tree [(*Psorothamnus spinosus* (Gray) Barneby = *Dalea spinosa* Gray] is a desert botanical species which has economic potential [10]. It is a shrub or small tree found in sandy arroyo habitats of the northwestern Sonoran Desert of southwestern North America. The calyx or pericarp of the seed pods have resin sacs which have potential as a source of aromatic components for use in the cosmetic industry. The seeds also contain a triglyceride-based oil with a unique fatty acid profile which may have potential as a cosmetic or specialty food ingredient, although the low percentage of this oil in the seed (3-4%) may limit its potential.

The reported extractions of *Dalea* species have employed organic solvents, such as benzene, to recover coumarin-type compounds as well as

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pigments [11-13]. Petroleum ether has yielded extracts containing steroids, isoprenylflavanones, essential oils which are rich in mono- and sesquiterpenes, and chalcones [14-15]. In addition, carbohydrates have been isolated from the methanol extracts of *Dalea* species [14]. In this study, on-line and off-line SFE were coupled with supercritical fluid chromatography (SFC), gas chromatography (GC), and mass spectrometry (MS) to analyze the supercritical CO_2 and organic solvent extracts of *Dalea spinosa*, the desert smoke tree.

Individual parts of the plant seed were subjected to on-line SFE/SFC in order to ascertain compositional differences. In this case, on-line SFE was successfully used to simulate larger off-line SFEs, thereby allowing the SFE process to be optimized to yield the desired composition in the resultant extract. Hence, micro on-line SFE provided information as to the spatial location of desired components within the seed or plant matrix using minimal sample and suggested how the seed might be prepared for optimal and selective SFE. In addition, SFC allowed a comparison between off-line SFE and conventional organic solvent extraction with respect to efficacy of extraction.

EXPERIMENTAL

Materials and chemicals. The desert smoke tree and an isopropyl alcohol extract, referred to as "concrete," were provided by James H. Brown of International Flora Technologies (Apache Junction, AZ, USA). For the extractions, the plant components (petals, seeds, calyces, and stems) were either kept intact as received or ground up with a Model MC-170 Miracle Mill (Markson Science, Phoenix, AZ, USA). Carbon dioxide for the SFC and SFE/SFC runs was SFC-grade from Scott Specialty Gases (Plumsteadville, PA, USA). Welding-grade carbon dioxide, used in the off-line SFE experiments, was obtained in 60-lb, non-syphon tube cylinders, from National Welding Supply (Bloomington, IL, USA). Tenax-TA (Anspec, Ann Arbor, MI, USA) was the sorbent used to trap the volatile compounds for analysis during the off-line SFE experiments. The Tenax traps were conditioned at 200°C for 60 min with a helium flow rate of 40 mL min^{-1}.

SFE and SFC apparatus. Off-line SFE was performed on an apparatus previously described in the literature [16]. All SFC analyses were conducted on a Model 501 (Lee Scientific Division/Dionex Corporation, Salt Lake City, UT, USA) supercritical fluid chromatograph equipped with a flame ionization detector. Dynamic-split injection, using a 15-μm i.d. fused silica capillary as the split-line restrictor, was applied during the SFC runs. Three different fused-silica capillary columns (10 m x 0.1 mm i.d.) from Lee Scientific were utilized in these studies: SB-Phenyl-5, SB-Phenyl-50, and SB-Octyl-50; all having a film thickness of 0.5 μm.

The following pressure program was used for analyses with the SB-Phenyl-5 column: 100 atm for 20 min, ramped at 3 atm min^{-1} to 220 atm, then 0.75 atm min^{-1} to 240 atm, then 5 atm min^{-1} to 350 atm, and held for 5 min. The oven temperature was maintained at 75°C for 20 min and then raised at 50°C min^{-1} to 100°C and held there for the duration of the chromatographic run. The pressure program for the SB-Phenyl-50 column was 100 atm for 15 min, ramped at 3 atm min^{-1} to 200 atm and held for 5 min, increased at 0.25 atm min^{-1} to 205 atm, then 3 atm min^{-1} to 295 atm, then 0.5 atm min^{-1} to 302 atm, then 5 atm min^{-1} to 325 atm where it was held for 5 min. The oven temperature was held at 100°C for the entire run. The SB-Octyl-50 column pressure program consisted of 150 atm for 12 min, increased at 2 atm min^{-1} to 190 atm and held for 5 min, then 2 atm min^{-1} to 310 atm and held for 5 min. The oven temperature was 100°C.

On-line SFE/SFC experiments employed a modified Brownlee NewGuard column and its holder (Applied Biosystems, Foster City, CA, USA) as the micro-extraction cell. Extractions were performed with the micro-extractor fitted on the Model 501 SFC. The extract was concentrated in the cryofocusing trap, which was cooled with industrial grade CO_2, before chromatography. Deactivated frit restrictors obtained from Lee Scientific were used to maintain the column pressure. The detector temperature was held at 350°C. The experimental conditions for SFE differed according to the sample requirements, and these are cited where appropriate in the text.

Gas chromatography/mass spectrometry. A Model 3400 (Varian Associates, Sunnyvale, CA, USA) gas chromatograph fitted with a Durabond-1701 (J&W Scientific, Folsom, CA, USA) capillary column (30 m x 0.32 mm i.d., 0.25 μm film thickness) was connected to an Incos 50 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA). Headspace GC/MS data were acquired with a Model 4000 Tekmar Concentrator (Tekmar, Cincinnati, OH, USA) attached to a GC/MS system (Perkin Elmer Sigma 3B GC, Norwalk, CT, USA/Finnigan OWA 1050 MS).

Gas chromatography was performed using a programmed temperature run starting at 0°C for 5 min. The oven temperature was then ramped to 250°C at a rate of 5°C min^{-1}. The injection temperature was 150°C. Conditions for the purge-and-trap/headspace analysis were purge rate of 30°C min^{-1} followed by trapping at 25°C; approximately 150°C desorption temperature for the Tenax-TA trap; and 25°C trapping temperature to avoid thermal decomposition/rearrangement of the extracted analytes. Volatile compounds were tentatively identified employing a Wiley/NBS mass spectral library and an in-house MS database stored on a Modcomp computer. Confirmation of several compounds was accomplished by comparing spectra and retention times of standards.

RESULTS AND DISCUSSION

The objective of this study was to demonstrate the viability of SFE and SFC as techniques for characterizing the described desert botanical species. The whole plant as well as the individual parts were investigated for their compositional differences by on-line SFE/SFC. To optimize extraction efficiencies, the extraction pressure was varied while holding the temperature constant at 45°C to avoid thermal degradation of the volatile compounds. The pressures used were 100, 200, 300, and 400 atm. Extraction at 200 atm provided good recoveries without overloading the capillary column. Furthermore, extraction at 200 atm did not yield an over-abundance of material that contaminated the cryofocusing trap and associated plumbing so that an excessive number of blank extractions had to be performed to clean the system. Also, the length of the extraction time was kept short (1 min) for the above reasons.

All of the *D. spinosa* on-line SFE/SFC trials were non-exhaustive extractions because of the susceptibility to overload the SFC capillary column. When a second SFE/SFC experiment was performed on the same extracted seed, the chromatographic profile showed more detector response. This is probably due to the seed becoming fractured during the initial SFE, thus allowing more efficient mass transfer of the solutes from the seed.

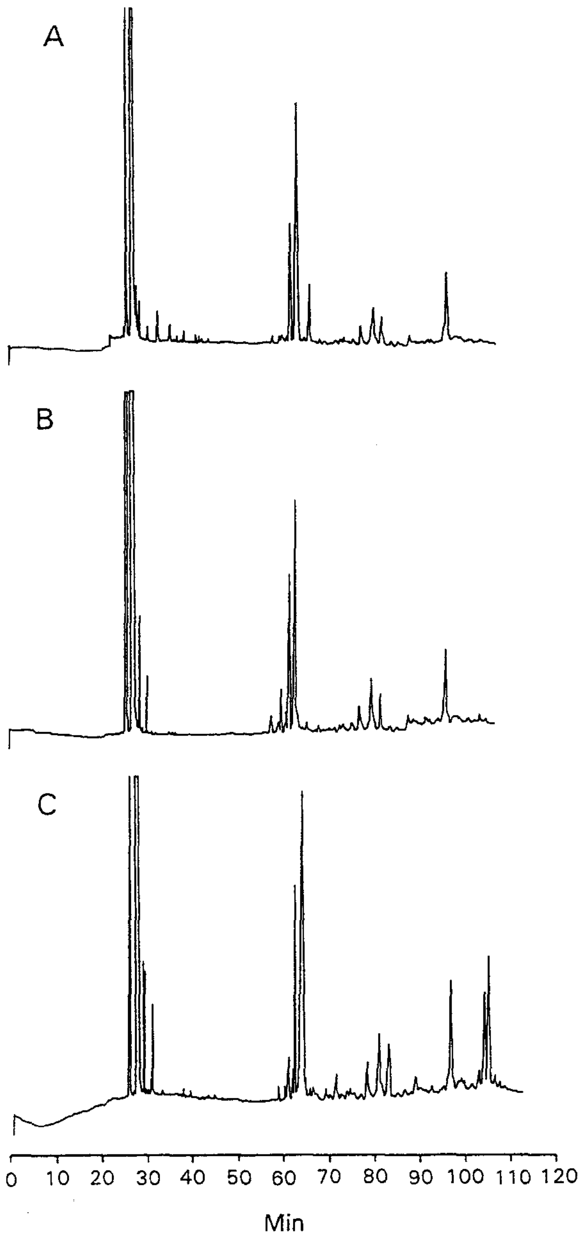

Figure 1. *SFC chromatograms of Dalea spinosa extracts using an SB-Phenyl-5 capillary column: (A) isopropyl alcohol extract, (B) SFE of intact sample, and (C) SFE of ground sample.*

The above observation was supplemented by off-line SFE experiments run at two different sets of pressures and temperatures (204 atm at 45°C and 680 atm at 80°C). The first set of conditions simulated the on-line SFE runs, while the second set of conditions was chosen based on our previous studies on exhaustive delipidation of oilseeds. The first extraction under each set of conditions was performed on the intact *Dalea spinosa*. The sample was then removed from the extraction cell and ground to a powder. This comminuted material was reextracted under the same conditions, resulting in more extract being recovered. This enhanced recovery was experienced with both sets of extraction conditions.

The initial extraction yield at 204 atm and 45°C was 2.9 wt%. The second SFE (on the ground matrix) more than doubled the initial recov-

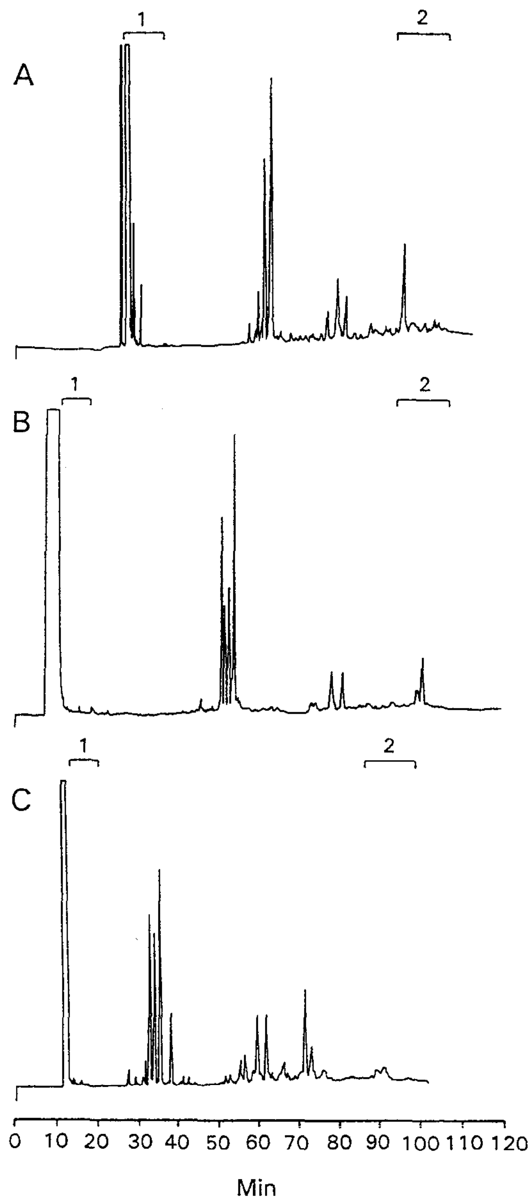

Figure 2. *SFC chromatograms showing the effect of stationary phase selectivity on the separation of components of a Dalea spinosa extract. See text for explanation.*

ery, yielding 7.3 wt%. For SFE at 680 atm and 80°C, the initial yield was 6.5 wt%. An additional 7.7 wt% of the extract was recovered after the second extraction. The increased recovery after grinding of the sample is similar to the findings of Snyder *et al.* [17] for oil recovery from vegetable seeds. In addition, a smoke tree sample that had been extracted with isopropyl alcohol was ground and reextracted with supercritical CO_2 at 680 atm and 80°C. Again, SFE yielded an extra 5.5 wt% of the extract, showing that organic solvent extraction of the intact material was not complete.

SFC comparison of the above off-line supercritical CO_2 extracts using two different sets of extraction conditions gave very similar chromatographic profiles. The only major difference was the relative amounts of triglyceride peaks. The extract from the SFE conducted at 680 atm and 80°C showed an enrichment in triglyceride-based peaks. This result is consistent with previous studies, showing that exhaustive lipid extraction can best be conducted at higher pressures and temperatures [18].

One of the objectives of this study was to determine whether or not extraction of *D. spinosa* with supercritical CO_2 would yield an extract equivalent to an isopropyl alcohol-derived concrete. Figure 1 shows supercritical fluid chromatograms, performed under identical chromatographic conditions using an SB-Phenyl-5 capillary column, of an isopropyl alcohol extract, a supercritical CO_2 extract of a neat sample, and a supercritical CO_2 extract of a ground sample. The SFC results in Figure 1 show that a comparable extract can be produced by the SFE process, with an exception being that the ground matrix was enriched in the late-eluting glyceride components [19]. Also, a peak was noted in the isopropyl alcohol extract (at *ca.* 65 min) that was significantly less in the supercritical CO_2 extracts.

Optimizing SFC resolution can be a complex process when chromatographing an extract containing many unidentified components. Column type, oven temperature, pressure, and/or density programming are all parameters that must be investigated. In these studies, capillary SFC was attempted on three columns having different stationary phases (Figure 2). Each column had both positive and negative characteristics regarding peak resolution and compound response. The SB-Phenyl-5 column showed the best resolution of the early eluting volatile peaks, but poorer resolution of the late eluting peaks (Figure 2A). The SB-Phenyl-50 column (Figure 2B) did not resolve the early eluting peaks; however, the SB-Phenyl-50 provided enhanced resolution of the peaks eluting in the middle of the chromatogram, relative to the SB-Phenyl-5 column results. Finally, the SB-Octyl-50 column (Figure 2C) exhibited the best resolution for the late eluting peaks. Resolution of the early eluting volatile compounds was rather poor due to overlap with the solvent peak. The peaks eluting in the middle of the chromatogram exhibited good resolution.

Experiments were also run to compare the supercritical fluid chromatograms of the extracts produced from the on-line and off-line extractions

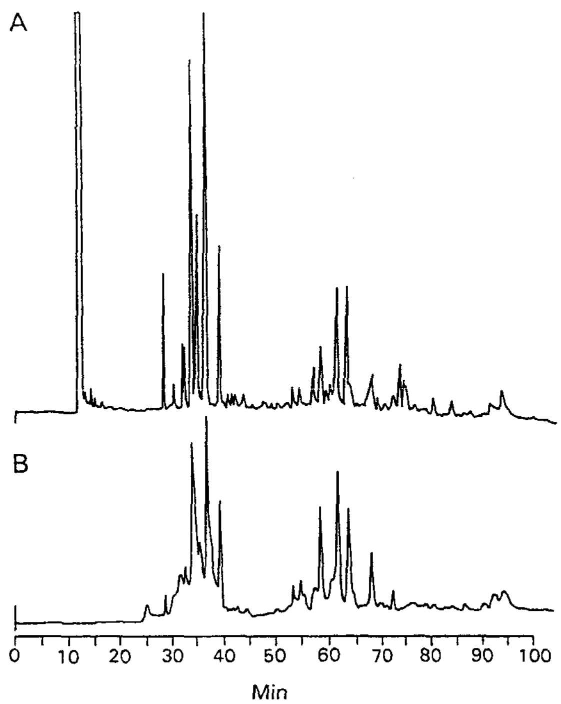

Figure 3. *SFC comparison of off-line SFE (A) to on-line SFE (B) of Dalea spinosa using an SB-Octyl-50 capillary column.*

conducted at 200 atm and 45°C. Figure 3 shows that the chromatograms are very similar with regard to the major component peaks in the respective extracts, although there is evidence of column overload in the on-line SFE/SFC experiment. Caution should be exercised in extrapolating results obtained from the small sample sizes normally associated with on-line SFE studies because of sample inhomogeneity. However, the results in Figure 3 demonstrate that on-line SFE can be used to predict results obtainable by larger scale off-line SFE. This also permits the optimization of SFE with respect to a particular extract composition.

Micro-SFE coupled with analytical chromatography can also be used as a versatile technique for examining the composition and extractability of component parts of plants. In Figure 4, supercritical fluid chromatograms were obtained by performing on-line SFE under identical conditions (200 atm at 45°C for 1 min with cryofocusing) on a whole seed of *Dalea spinosa*, its calyx, and a resin sac. The resin sac was excised from the calyx using a surgical scalpel. The chromatograms for these plant parts are quite different as shown in Figure 4, under identical SFC conditions (SB-Octyl-50 column, using parameters previously stated). It is apparent that the resin sac is enriched in the lower molecular weight compounds and that

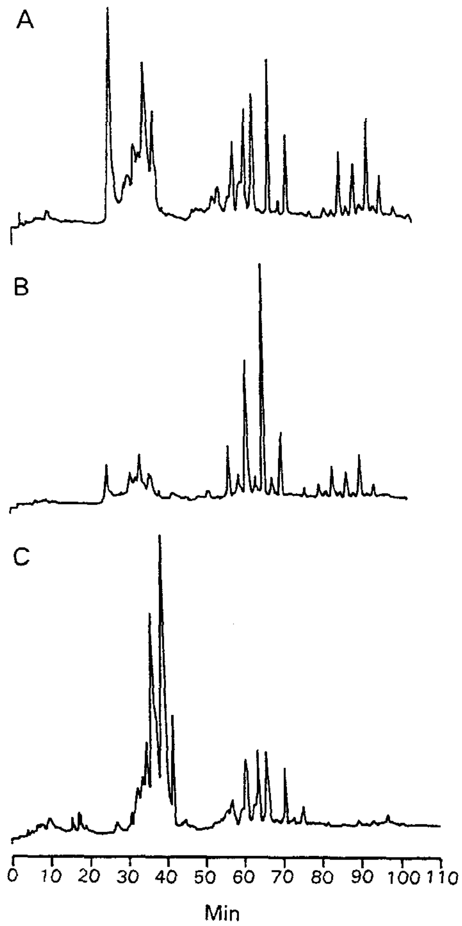

Figure 4. *On-line SFE/SFC comparison of Dalea spinosa components: (A) seed, (B) calyx, and (C) resin sac.*

the whole seed is enriched in triglycerides (late eluting peaks). SFE/SFC of the calyx reveals an extract largely devoid of lower molecular weight compounds and enriched in higher molecular weight components. This result indicates that the resin sac, as opposed to the calyx, is the primary source of the volatile odoriferous components. These results provide vital information as to the spatial location of various components within the plant, and suggest how the plant matrix might best be prepared for selective SFE [20].

SFC/MS analyses have been conducted on plant extracts [21], but such equipment was not available to us at the time of these studies. However, GC/MS studies were conducted on headspace samples and extracts of the desert smoke tree obtained by SFE and isopropyl alcohol extraction. A sorbent trap of Tenax-TA was used for collecting volatiles during the off-line SFE experiments. The volatiles were thermally desorbed onto a GC column and analyzed by GC/MS (Figure 5). The

Table I. *Compounds found in D. spinosa SFE extracts.*[a]

Headspace GC/MS
α-Pinene[b]
β-Pinene[b]
β-Myrcene[b]
Limonene[b]
4-Carene[b]
Cineole
Octyl acetate
4,5-dimethyl-1-hexene
Propanoic acid, 2-methyl-,2-methylpropyl ester
Propanoic acid, 2-methyl-,2-methylbutyl ester
Butanoic acid, 3-methyl-,3-methylbutyl ester
GC/MS
Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-benzopyran-8-y1)-[c]
Ethanone, 1-(3,4-dihydro-7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-benzopyran-8-y1)-
Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-1,1,4a-trimethyl-8-isopropyl

[a]Identifications are based on computer library searches and are tentative.
[b]Identification was made by comparing spectra and retention times with those of standard compounds.
[c]Alloevodionol is the common name.

GC was programmed to analyze even the most volatile peaks. As shown in Table I, many of the volatile compounds are those commonly found in essential oils.

Also listed in Table I are three of the six major non-volatile components in the *D. spinosa* extracts identified by direct injection GC/MS. Two of the components have been tentatively identified as alloevodionol, a natural chromene first isolated by Sutherland [22], and its hydrogenated analog. The third compound has been tentatively identified as a possible precursor of 12-hydroxytotarol which is implicated as a biogenic precursor of nagilactones. Nagilactones have been found to exhibit a wide range of biological properties including antitumor activity as well as potential as plant growth regulators [23]. These three compounds were identified by library spectral matches from the Incos 50 and mainline Modcomp data systems. Both data systems produced the same tentative identifications for the compounds.

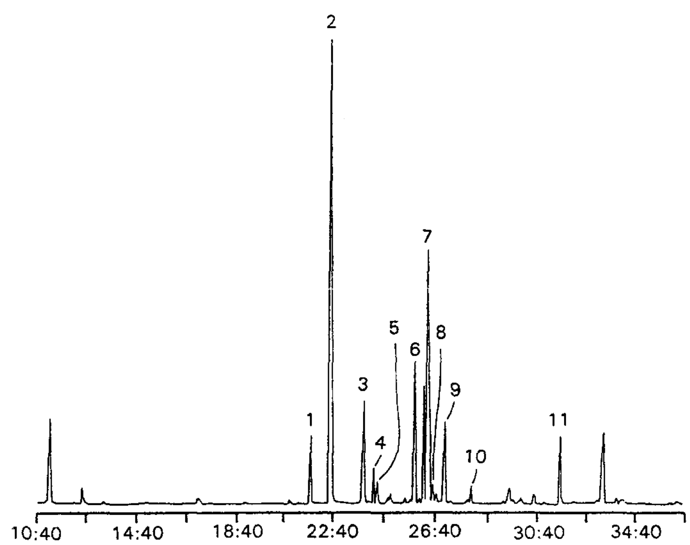

Figure 5. *Headspace GC/MS of volatiles from a Dalea spinosa supercritical fluid extract. See text for details. Peak identifications: (1) α-pinene, (2) propionic acid, 2-methyl-,2-methylpropyl ester, (3) β-pinene, (4) β-myrcene, (5) propionic acid, 2-methyl-,2-methylbutyl ester, (6) limonene, (7) butanoic acid, 2-methyl-,3 methylbutyl ester, (8) cineole, (9) 4-carene, (10) 4,5-dimethyl-1-hexene, (11) octyl acetate.*

It was demonstrated in this study that on-line SFE/SFC permits qualitative characterization of the extracts derived from the individual plant parts. This information can be employed to optimize conditions for larger commercial scale SFE, including the requisite physical configuration of the substrate. The micro SFE/SFC experiments indicate that the *D. spinosa* seed need not be crushed to extract the aromatic compounds responsible for its pleasant odor. These results show that comminution of the seed was deleterious to isolating a highly purified aroma concentrate for cosmetic purposes [24] due to contamination by coextracted lipids.

ACKNOWLEDGMENT

The assistance and interest of Mr. James H. Brown of International Flora Technologies is gratefully appreciated. We also acknowledge the assistance of Dr. Henry Rakoff with the interpretation of the mass spectra.

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Received: June 16, 1994
Accepted: August 16, 1994